

Patent
Attorney Docket: 612,404-387
(Former L&L Ref: 267/242)

Amendments to the claims:

Please amend the claims as follows:

1. (Currently amended) A method for the amplification of a nucleic acid sequence, comprising the steps of:
 - providing a target nucleic acid sequence;
 - providing a first polynucleotide sequence having at least one donor chromophore, the first polynucleotide sequence being complementary to at least a portion of the target nucleic acid sequence;
 - providing a second polynucleotide sequence having at least one acceptor chromophore, the second polynucleotide sequence being complementary to at least a portion of the target sequence;
 - performing polymerase chain reaction to amplify the target nucleic acid sequence;
 - hybridizing the first and second polynucleotide sequences to the target nucleic acid sequence, such that when the first polynucleotide sequence and the second polynucleotide sequence are hybridized to the target nucleic acid sequence, the donor chromophore and acceptor chromophore are in an energy transfer relationship that substantially eliminates quenching; and
 - irradiating the mixture to detect hybridizations of the first and second polynucleotide sequences by fluorescence energy transfer from the one or more donor chromophores of the first polynucleotide sequence to the one or more acceptor chromophores of the second polynucleotide sequence.
2. (Original) The method of claim 1, wherein the reaction mixture is a homogeneous format.
3. (Original) The method of claim 1, wherein the target nucleic acid comprises double stranded or denatured DNA.
4. (Currently Amended) The method of claim 1, wherein the mixture is irradiated ~~amplification is monitored~~ for each PCR cycle.
5. (Original) The method of claim 1, wherein the target nucleic acid sequence

Patent
Attorney Docket: 612,404-387
(Former L&L Ref: 267/242)

comprises RNA.

6. (Original) The method of claim 1, wherein the target nucleic acid sequence comprises synthetic polynucleotide.

7. (Original) The method of claim 1, wherein the hybridization and amplification reactions occur entirely in solution.

8. (Original) The method of claim 1, wherein at least one of the first polynucleotide, the second polynucleotide, or the target nucleic acid is bound to a solid support or matrix that is insoluble in the analyte solution.

9. (Original) The method of claim 8, wherein the solid support or matrix is selected from the group consisting of glass, metals, silicon, organic polymers, membranes, and biopolymers.

10. (Original) The method of claim 1, wherein the polynucleotide sequence comprises a plurality of donor chromophores.

11. (Original) The method of claim 1, wherein the polynucleotide sequence comprises a plurality of acceptor chromophores.

12. (Currently Amended) The method of claim 1, further comprising the step of hybridizing another first polynucleotide sequence and another second polynucleotide sequence to the target nucleic acid sequence, wherein the donor chromophore and acceptor chromophore are in an energy transfer relationship. ~~wherein the step of hybridizing the first and second polynucleotide sequences to the target nucleic acid sequence takes place during a PCR cycle.~~

13. (Currently Amended) The method of claim 12, further comprising the step of irradiating the mixture to detect hybridization of said another first and second polynucleotide sequences by fluorescence energy transfer from the one or more donor chromophore of said another first polynucleotide sequence to the one or more acceptor chromophore of said another second polynucleotide sequence. ~~1, wherein the step of irradiating the mixture to detect hybridizations of the first and second polynucleotide sequences by fluorescence energy transfer from the one or more donor chromophores of the first polynucleotide sequence to the one or more acceptor chromophores of the second polynucleotide sequence takes place during a PCR cycle.~~

Patent

Attorney Docket: 612,404-387

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14. (Original) The method of claim 1, wherein each of the donor chromophores and the acceptor chromophores is selected from the group consisting of 4,4'-Diisothiocyanatodihydrostilbene-2,2'-disulfonic acid, 4-acetamido-4'-isothiocyanato-stilbene-2,2'-disulfonic acid, 4,4'-Diisothiocyanatostilbene-2,2'-disulfonic acid, Succinimidyl pyrene butyrate, Acridine isothiocyanate, 4-methylaminophenylazophenyl-4'-isothiocyanate (DABITC), Lucifer Yellow vinyl sulfone, Fluorescein isothiocyanate, Reactive Red 4 (Cibacron Brilliant Red 3B-A), Rhodamine X isothiocyanate, Texas Red (Sulforhodamine 101, sulfonyl chloride), Malachite Green isothiocyanate, or IR144.

15. (Original) The method of claim 1, wherein the step of providing a target nucleic acid sequence further comprises the steps of providing an mRNA sequence producing a double-stranded cDNA sequence from the mRNA sequence.

Patent
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16. (Currently Amended) A method for the amplification of a nucleic acid sequence, comprising the steps of:
- providing a target nucleic acid sequence;
 - providing a first polynucleotide sequence having at least one donor chromophore, the first polynucleotide sequence being complementary to at least a portion of the target nucleic acid sequence;
 - providing a second polynucleotide sequence having at least one acceptor chromophore, the second polynucleotide sequence being complementary to at least a portion of the target sequence;
 - providing four different nucleoside triphosphates, a thermostable amplification enzyme, and two primers, wherein the primers are substantially complementary to the target polynucleotide sequence;
 - denaturing the target nucleic acid sequence to form single stranded nucleic acids at an appropriate temperature;
 - hybridizing the first and second polynucleotide sequences to the target nucleic acid sequence, such that when the first polynucleotide sequence and the second polynucleotide sequence are hybridized to the target nucleic acid sequence, the donor chromophore and acceptor chromophore are in an energy transfer relationship that substantially eliminates quenching; and
 - irradiating the mixture to detect hybridizations of the first and second polynucleotide sequences by fluorescence energy transfer from the one or more donor chromophores of the first polynucleotide sequence to the one or more acceptor chromophores of the second polynucleotide sequence; and
 - elongating the target polynucleotide sequence by adding the nucleotide triphosphates. ~~elongating of the target polynucleotide sequence under conditions that the target polynucleotide sequence is amplifiable.~~

17. (Original) The method of claim 15, wherein the target nucleic acid comprises double stranded DNA.

18. (Original) The method of claim 15, wherein the target nucleic acid sequence comprises synthetic polynucleotide.

Patent
Attorney Docket: 612,404-387
(Former L&L Ref: 267/242)

19. (Original) The method of claim 16, wherein the hybridization and amplification reactions occur entirely in solution.
20. (Original) The method of claim 16, wherein at least one of the first polynucleotide, the second polynucleotide, or the target nucleic acid is bound to a solid support or matrix that is insoluble in the analyte solution.
21. (Original) The method of claim 20, wherein the solid support or matrix is selected from the group consisting of glass, metals, silicon, organic polymers, membranes, and biopolymers.
22. (Original) The method of claim 16, wherein the polynucleotide sequence comprises a plurality of donor chromophores.
23. (Original) The method of claim 16, wherein the polynucleotide sequence comprises a plurality of acceptor chromophores.
24. (Original) The method of claim 16, wherein the step of providing a target nucleic acid sequence further comprises the steps of providing an mRNA sequence producing a double-stranded cDNA sequence from the mRNA sequence.